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GROWTH AND TOXICITY OF *PRYMNESIUM PARVUM* (HAPTOPHYTA) AS A FUNCTION OF SALINITY, LIGHT, AND TEMPERATURE[1]

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The growth rate, stationary cell concentration, and toxicity of *Prymnesium parvum* N. Carter were measured using a strain isolated from Texas inland waters. We used a multifactor experimental approach with multiple regression analysis to determine the importance of environmental factors, including temperature, light, and salinity to these algal measurements. Exponential growth rate was unimodal in relation to temperature, salinity, and irradiance, with an estimated maximal growth of $0.94\,d^{-1}$ occurring at 27°C, 22 practical salinity units (psu), and 275 µmol photons $\cdot m^{-2} \cdot s^{-1}$. Stationary cell concentrations also had unimodal responses to temperature and salinity but increased with irradiance. Maximal cell concentrations were estimated to occur at 26°C and 22 psu. Both maximum growth rate and highest stationary cell concentrations were measured at levels of each factor resembling warm, estuarine conditions that differ from the conditions under which blooms occur in inland waters in the southwestern United States. Acute toxicity to fish was highest at the lowest salinity and temperature levels, conditions not optimal for exponential growth but similar to those under which blooms occur in inland waters. Our results imply that summer blooms could occur in inland waters of the southwestern United States. Generally, they have not, suggesting that factors other than those investigated in this research influence bloom dynamics.

Key index words: **algal toxicity; niche factors; population dynamics; *Prymnesium parvum***

Abbreviations: **LC_{50}, 50% lethal concentration; USEPA, U.S. Environmental Protection Agency**

Interest in the ecology of harmful algal blooms (HABs) has increased in the recent past due to their apparently increasing incidence worldwide (Hallegraeff 1993). *Prymnesium parvum* forms harmful blooms, first attracting attention after fish kills in Europe (Otterstrøm and Steeman Nielsen 1940) and Israel (Reich and Aschner 1947). *Prymnesium parvum* and similar species have a widespread geographic distribution (Moestrup 1994, Edvardsen and Paasche 1998). Blooms of *Prymnesium* species have occurred in coastal waters of Norway (Johnsen and Lein 1989), China (Guo et al. 1996), Holland (Shilo and Shilo 1953), Spain (Comin and Ferrer 1978), and many other regions worldwide (Edvardsen and Paasche 1998). Blooms of *P. parvum* in inland waters also occur in Texas and other parts of the southwestern United States. The tendency of *P. parvum* to form HABs in widespread locations motivates the study of the ecological factors that affect its growth and abundance.

Confirmed *P. parvum* blooms in Texas have been documented since 1985 (Texas Parks & Wildlife 2003). Blooms have caused fish kills in 19 reservoirs along major river systems, including the Brazos, Rio Grande, Colorado, and Red rivers. *Prymnesium parvum* blooms in reservoirs have resulted in some 17.5 million fish killed and an estimated economic impact in the tens of millions of dollars as of 2003 (Texas Parks & Wildlife 2003). Confirmed blooms can occur anytime in the

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year, but most of the blooms in Texas have occurred in fall to spring months with the majority between October and April (J. Glass, personal communication). These months are cool compared with the summer in Texas, suggesting that temperature is an important niche factor for *P. parvum*. In addition, the amount of light received in Texas reservoirs varies with season, and salinity varies with location. The effects of temperature, light, and salinity on exponential growth rates have been studied for several strains of *P. parvum* and *P. patelliferum* (Table 1), which appears to be a form of the same species (Larsen and Edvardsen 1998). Where information on origin is available, these previously studied strains were derived from marine waters. Little similar information is known for strains of *P. parvum* derived from inland waters.

Prymnesium parvum is a unicellular haptophyte, with two flagella for locomotion and an additional anterior appendage, the haptonema (Lee 1980). Cells are highly motile and have been reported to survive and bloom in a variety of environmental conditions, though mostly in inshore, brackish waters (Moestrup 1994). *Prymnesium parvum* is mixotrophic, ingesting bacteria, protists, and other algae in addition to producing energy through photosynthesis (Tillman 1998, Legrande et al. 2001, Skovgaard et al. 2003).

Prymnesium parvum is believed to produce a number of toxins. Unfortunately, quantification of these toxins remains difficult. Historically, toxicity has been estimated by various measures of ichthyotoxic, neurotoxic, cytotoxic, and hemolytic activities (Shilo 1981). *In vitro* hemolytic assays have recently become common (Johansson and Granéli 1999, Barreiro et al. 2005), as have assays with invertebrates such as *Artemia* spp. (Larsen et al. 1993, Larsen and Bryant 1998). Although much recent concern arises from fish kills associated with *P. parvum* blooms, and the earliest studies of toxicity used fish (genus *Gambusia*, Shilo and Aschner 1953), most recent studies have not assessed toxicity to fish. Toxicity, assessed by various other measures, is influenced by temperature, salinity, and irradiance (Shilo 1981, Larsen et al. 1993, Larsen and Bryant 1998) and also increases when cells are grown under nutrient deficiency (Dafni et al. 1972, Larsen et al. 1993, Granéli and Johansson 2003). Two of the toxins produced by *P. parvum* have been characterized chemically, prymnesins 1 and 2 (Igarashi et al. 1996), though these compounds are difficult to study (Murata and Yasumoto 2000), and the presence of other toxins has not been ruled out. These difficulties, combined with the relative scarcity of studies directly addressing toxicity to fish, prompted us to examine acute toxicity to fish in relation to temperature, salinity, and irradiance.

This study reports laboratory culture experiments designed to define the ecological response and toxic potential of *P. parvum* in relation to temperature, light, and salinity, using a strain isolated from inland waters in Texas. The experimental design was a multifactor response surface design (Cochran and Cox 1957) addressing two important possibilities in niche studies: curvilinear responses, such as saturating or unimodal responses, and interactions among multiple factors. Such experimental designs address complex niche responses while using the fewest experimental units possible. Despite the long-standing availability of such experimental designs and their high efficiency, they are not commonly used in niche studies of algae. Of the studies that analyzed the effects of growth factors on the exponential growth of *Prymnesium* (Dickson and Kirst 1987, Larsen et al. 1993, Larsen and Bryant 1998), only one used combinations of two niche factors (Larsen et al. 1993), although interactions were not analyzed statistically and none used combinations of three factors (Table 1). The data from response surface designs are ideal for regression analysis and here are used to develop predictive regression models to further the understanding of toxic blooms under varying environmental conditions.

MATERIALS AND METHODS

Strain history and culturing. The *P. parvum* culture used in this study was provided from the UTEX (University of Texas at Austin) Culture Collection of Algae (UTEX LB ZZ181). This strain was isolated from a bloom dominated by *P. parvum* and causing an active fish kill in the Colorado River (J. Glass, personal communication). Identification and classification of *P. parvum* was accomplished using gene sequence analysis and SEM (J. Glass, personal communication) and confirmed by investigators at the USDA, the University of Texas, and Woods Hole Oceanographic Institute as *P. parvum* (J. Glass, personal communication).

Stock cultures were grown in a defined medium of artificial seawater (Kester et al. 1967), diluted to 5.8 psu salinity in $18\,M\Omega \cdot cm^{-1}$ ultrapure water (Millipore Corp., Bedford, MA, USA) and enriched with f/2 levels of nitrogen, phosphorus, trace metals, and vitamins (MacLachlan 1973). In the trace metals solution, an equimolar amount of ferric chloride was substituted for ferrous ammonium sulfate. These stock cultures were maintained in an incubator at 20°C and on a 12:12 light:dark (L:D) photoperiod with an irradiance of ~150 μmol photons $\cdot m^{-2} \cdot s^{-1}$. This photoperiod was used for all cultures in this study because blooms typically begin in Texas at approximately this photoperiod in fall and early winter. For experimental cultures, different incubators (Model CEL 511-38, Sherrer-Gillett Co., Marshall, MI, USA; Model I36LLVLC8, Percival Scientific Inc., Perry, IA, USA) were used to achieve different temperatures. Shrouds made with shade cloth, the number of lamps (Simkar 40 W, Philadelphia, PA, USA) in an incubator, and changes in flask position were used as needed to adjust irradiance to experimental levels. The irradiance of experimental cultures was measured using a photon flux meter (Model LI-1400, LI-COR, Lincoln, NE, USA) positioned at the sides and above and below several culture vessels. Such measurements were taken in several areas of the incubator containing experimental cultures and then averaged to obtain the reported irradiance of each experimental level.

Stock and experimental cultures were not axenic. Maximum bacteria cell concentrations in experimental cultures reached 5.2×10^7 cells $\cdot mL^{-1}$, though most cultures were below this level with an average of 1.14×10^7 cells $\cdot mL^{-1}$.

For the experimental cultures, salinity was controlled by changing the proportion of artificial seawater to ultrapure water ($18\,M\Omega \cdot cm^{-1}$, Millipore). After dilution to the experimental salinity, nutrients ($NaNO_3$, NaH_2PO_4, vitamins, trace

TABLE 1. Previous studies of temperature, salinity, and irradiance effects on the exponential growth rate of *Prymnesium*.

Strain	Source	Temperature	Salinity	Irradiance	Reference
Prymnesium parvum K0081	Denmark (56° N)	Unimodal over 10°C–28°C, optimum of ~0.8 d^{-1} at 26°C	Growth more rapid at 8 and 25 psu than at 2 and 34 psu	Increasing over 40–500 μmol photons · $m^{-2} \cdot s^{-1}$, saturating at about 200 μmol photons · $m^{-2} \cdot s^{-1}$	Larsen et al. (1993)
Prymnesium parvum K0081	Denmark (56° N)	Unimodal over 5°C–30°C, optimum of ~0.4 d^{-1} at 15°C	Unimodal over 3–30 psu, optimum of about 0.7 d^{-1} at 18 psu	Increasing over 25–250 μmol photons · $m^{-2} \cdot s^{-1}$, saturating at about 150 μmol photons · $m^{-2} \cdot s^{-1}$.	Larsen and Bryant (1998)
Prymnesium patelliferum J. C. Green, D. J. Hibbered et R. N. Pienaar K0252	Australia (38° S)	Increasing over 13°C–28°C, maximum of ~1.0 d^{-1}		Increasing over 40–500 μmol photons · $m^{-2} \cdot s^{-1}$, saturating at about 200 μmol photons · $m^{-2} \cdot s^{-1}$.	Larsen et al. (1993)
Prymnesium patelliferum N	Norway (59° N)	Increasing over 10°C–28°C, maximum of about 0.8 d^{-1}	Growth more rapid at 8–34 psu than at 2 psu	Increasing over 40–500 μmol photons · $m^{-2} \cdot s^{-1}$, saturating at about 100 μmol photons · $m^{-2} \cdot s^{-1}$	Larsen et al. (1993)
Prymnesium parvum B127.79=PLY94	United Kingdom (ca. 52° N)		Increasing over 7–32 psu, maximum of about 0.8 d^{-1}		Dickson and Kirst (1987)
Prymnesium parvum PLY94	United Kingdom (52° N)	Unimodal over 5°C–30°C, optimum of about 0.2 d^{-1} at 15°C	Increasing over 3–30 psu, maximum of about 0.2 d^{-1}	Increasing over 25–250 μmol photons · $m^{-2} \cdot s^{-1}$, saturating at about 100 μmol photons · $m^{-2} \cdot s^{-1}$	Larsen and Bryant (1998)
Prymnesium patelliferum PLY527	United Kingdom (51° N)	Increasing over 5°C–30°C, maximum of about 0.5 d^{-1}	Increasing over 3–30 psu, maximum of about 0.7 d^{-1}	Increasing over 25–250 μmol photons · $m^{-2} \cdot s^{-1}$, saturating at about 150 μmol photons · $m^{-2} \cdot s^{-1}$	Larsen and Bryant (1998)
Prymnesium patelliferum RHpat93	Norway (59° N)	Unimodal over 5°C–30°C, optimum of about 0.5 d^{-1} at 15°C	Unimodal over of 3–30 psu, optimum of about 0.7 d^{-1} at 8 psu	Increasing over 25–250 μmol photons · $m^{-2} \cdot s^{-1}$, saturating at about 100 μmol photons · $m^{-2} \cdot s^{-1}$	Larsen and Bryant (1998)
Prymnesium parvum RL10parv93	Norway (59° N)	Unimodal over 5°C–30°C, optimum of about 0.4 d^{-1} at 15°C	Unimodal over 3–30 psu, optimum of about 0.5 d^{-1} at 8 psu	Increasing over 25–250 μmol photons · $m^{-2} \cdot s^{-1}$, saturating at about 150 μmol photons · $m^{-2} \cdot s^{-1}$	Larsen and Bryant (1998)
Prymnesium parvum (Droop's)	Unknown		Unimodal over 0–45 psu, optimum of about 1.0 d^{-1} at 15 psu		Brand (1984)
Prymnesium parvum (McCarthy's)	Unknown		Unimodal over 2–33 psu, optimum of about 0.8 d^{-1} at 19 psu; optimum of about 0.7 d^{-1} at 25 psu		Padilla (1970)

metals) were added at f/2 concentrations, and bicarbonate was added at 93 μM. The $NaNO_3$ was added before autoclaving, but the other nutrients were filter sterilized (Nalgene, 0.2 μm, 25 mm syringe filters, Rochester, NY, USA) and added aseptically after sterilization to minimize precipitation. One-liter flasks were filled to a working volume of 600 mL, and each flask was inoculated with 100 cells · mL^{-1} of *P. parvum* from stock cultures in late exponential phase, grown as described. Flasks were then distributed to combinations of irradiance, temperature, and salinity in the experimental design described below. Flasks were mixed daily by gently swirling, and positions within incubators were rotated regularly.

Sampling and analysis. Samples of experimental batch cultures were taken on days 1–4, 7, 9, 11, 14, 17, 19, 21, and 23, until cultures reached stationary phase. At each sampling, aliquots of 5 mL were preserved with 0.15 mL Lugol's iodine for cell counts (modification of Throndsen 1978). Aliquots of 10 mL were preserved with 0.5 mL formalin for bacterial counts. Cell concentration was obtained by direct microscopic counts, using sedimentation chambers (Utermohl 25 mm, PhycoTech Inc., St. Joseph, MI, USA) and inverted microscopy (Olympus IMT-2, Olympus America Inc., Center Valley, PA, USA) for *P. parvum* (Margalef 1969, Booth 1993), and epifluorescence microscopy (Olympus BX40) for bacterial cells stained with acridine orange and collected on polycarbonate filters (Type GTBP 0.2 μm filters, Millipore Inc., Billerica, MA, USA) (Hobbie et al. 1977). Exponential growth rate of *P. parvum* in each culture was calculated by regressing the natural logarithm of cell concentration against time for days 2–9. Stationary cell concentration was taken as the average for days 17 and 23.

Lack of standards for prymnesins and the possible presence of other toxins precluded direct chemical analysis of toxins produced by *P. parvum*. For this study, toxicological bioassays were performed with a model freshwater fish to characterize acute toxicity and provide a surrogate measure of bioavailable toxin levels excreted by *P. parvum* under varied experimental conditions. Data from 48 h survival of juvenile fathead minnows (*Pimephales promelas*) were collected generally following USEPA text method 2000.0 for assessing acute toxicity to juvenile *P. promelas* (USEPA 2002) and applied to samples taken from stationary cultures with high cell concentrations. The fish used were <48 h old at the start of the assays and were fed newly hatched *Artemia* nauplii 2 h before initiation of testing. Survival of the fish was tested in a 0.5 dilution series of whole culture water into control water at seven levels from 100% to 6.25%. For each test, two replicate chambers with five organisms per chamber were used to assess toxic activity. A total of 70 fish were used in the tests. Acute LC_{50} values in terms of the dilution of water from each experimental unit were estimated using Probit (Finney 1971) or Trimmed Spearman–Karber (Hamilton et al. 1977) methods, as appropriate for data properties. Each estimated LC_{50} was then multiplied by the stationary cell concentration of *P. parvum* to estimate acute LC_{50} as the concentration of cells that resulted in 50% mortality of the fish. Thus, acute (48 h) LC_{50} expressed as cell concentration is an inverse measure of toxic activity per cell and potentially an indirect measure of the concentration of bioavailable toxins produced per cell. These cell-based LC_{50} estimates are reported here to permit comparison of the toxicity of *P. parvum* among cultures with different cell concentrations.

Experimental design and statistical analysis. In order to detect both unimodal responses to niche factors and interactions among factors, a three-factor response surface experimental design was used (Cochran and Cox 1957). The three factors of temperature, salinity, and irradiance each had five levels (Table 2). The design was rotatable so that standard errors of the fitted regression responses would be equal in concentric rings from the "center-point" (i.e., the midpoint of all three factors). The resulting experimental combinations of niche factors are illustrated in Table 2. The design had 12 replicate cultures at the center-point. Two replicate cultures were used at the "factorial points," which were combinations of the three factors at levels above and below the center-point conditions, and the "star points," which had extreme levels of one factor combined with the midpoints of the other two factors. The number of incubators available (four) was smaller than the number of temperatures required (five), so the experiment was divided into two incomplete blocks orthogonal to experimental treatments (Cochran and Cox 1957).

Exponential growth rate (μm), stationary cell concentration (N), and acute toxicity to fish (LC_{50}) were analyzed using multiple regression. The independent variables of temperature, salinity, and irradiance were centered on their means (the experimental midpoints) to avoid colinearity. The full model was fitted first, consisting of all linear, quadratic, and linear interaction terms for the three experimental factors. Exploratory analysis indicated that transforming the temperature data produced better model fit. We used an equation representing an asymmetric unimodal response as follows:

$$T_{\text{transform}} = \exp\left(\frac{\theta(T-20)}{20}\right) \quad (1)$$

where T is temperature (°C) and θ is a transformation parameter. The value for parameter θ was chosen to maximize fit (R^2) of the full model. Owing to heteroscedasticity and skew, stationary cell concentrations were transformed to natural logarithms, a common practice for population densities (Turchin and Taylor 1992). Following transformations and estimation of the full model, a best subsets regression scheme was used to seek a simpler regression model with acceptable fit using Mallow's C_p statistic (Kleinbaum et al. 1998). The best subsets search was restricted to avoid models having higher-order terms without the corresponding linear terms. As block effects are random and have no clear source, the block term was not included during model selection, though variance due to block effects was estimated for the full model. In all cases, a parsimonious predictive model could be identified, simpler than the full model but with similar goodness of fit. Lack of fit was tested for this model by constructing a sum of squares for pure error from the variances of replicates (Neter and Wasserman 1974), and residual plots were examined for failures of the regression assumptions. Both Statistica (v. 5.1, Statsoft, Tulsa, OK, USA) and SAS (v. 9.1., SAS Institute Inc., Cary, NC, USA) were used for these analyses and produced consistent results.

RESULTS

Exponential growth rate. The exponential growth rate was a unimodal function of temperature (Fig. 1A). This response was asymmetric and was represented by transforming temperature according to equation (1) with parameter $\theta = 1.87$. Interaction terms were insignificant ($t_{30} < 0.79$, $P > 0.434$) and explained <1% of the variance in the exponential growth rate. This lack of interactions in the predictive model implies that the optimal temperature for exponential growth rate does not vary significantly with salinity or irradiance. The block term was statistically significant ($t_{32} = -3.52$, $P = 0.001$) and explained 5% of the variance in exponential growth rate. Although this result sug-

TABLE 2. Experimental design and results.

Sample ID (block)	Salinity (psu)	Temperature (°C)	Irradiance (μmol photons · $m^{-2} \cdot s^{-1}$)	Exponential growth rate (d^{-1})	Stationary cell concentration (1000 cell · mL^{-1})	Toxicity (1000 LC_{50}, cell · mL^{-1})
1 (1)	28	29	122	0.822	1033.6	104.9
2 (1)	7.5	29	122	0.635	721.6	40.2
3 (1)	7.5	29	122	0.642	809.5	58.1
4 (1)	28	29	122	0.916	1153.7	77.3
5 (1)	7.5	29	336	0.758	483.3	19.9
6 (1)	7.5	29	336	0.695	546.7	57.4
7 (1)	28	29	336	0.745	660.2	67.0
8 (1)	28	29	336	0.776	1091.0	103.3
9 (1)	28	11	122	0.305	140.8	24.9
10 (1)	28	11	122	0.285	99.9	11.6
11 (1)	7.5	11	122	0.345	318.9	21.4
12 (1)	7.5	11	122	0.381	300.2	17.5
13 (1)	7.5	11	336	0.356	180.1	31.8
14 (1)	28	11	336	0.300	39.9	3.88
15 (1)	7.5	11	336	0.333	129.4	21.9
16 (1)	28	11	336	0.353	38.9	3.44
17 (1)	17.8	20	217	0.727	866.3	58.0
18 (1)	17.8	20	217	0.754	897.4	79.3
19 (1)	17.8	20	217	0.696	838.7	79.4
20 (1)	17.8	20	217	0.749	1425.7	63.0
21 (1)	17.8	20	217	0.766	1569.7	48.9
22 (1)	17.8	20	217	0.789	1360.1	56.0
23 (1)	17.8	20	217	0.696	910.6	56.9
24 (1)	17.8	20	217	0.727	1153.4	54.7
25 (2)	0.5	20	217	0.156	3.9	NR
26 (2)	0.5	20	217	0.156	3.2	NR
27 (2)	35	20	217	0.640	641.6	37.1
28 (2)	35	20	217	0.622	645.3	34.8
29 (2)	17.8	20	217	0.650	1127.1	86.3
30 (2)	17.8	20	217	0.629	1228.5	61.8
31 (2)	17.8	20	217	0.687	1299.9	86.6
32 (2)	17.8	20	217	0.630	1380.6	52.6
33 (2)	17.8	20	14	0.241	413.4	48.1
34 (2)	17.8	20	14	0.117	10.7	NR
35 (2)	17.8	5	217	0.045	1.5	NR
36 (2)	17.8	5	217	0.048	1.3	NR
37 (2)	17.8	20	420	0.666	800.8	70.5
38 (2)	17.8	20	420	0.671	756.5	65.8
39 (2)	17.8	35	217	−0.080	1.3	NR
40 (2)	17.8	35	217	−0.032	1.0	NR
Average				0.510	627.2	51.6

For toxicity, NR are data not reported due to limitations of the LC_{50} assay at very low cell concentrations.

gests systematic errors, the source of such errors is unknown and thus cannot be incorporated in the predictive model.

The predictive model for exponential growth rate had six terms, including all linear and quadratic terms as follows:

$$\begin{aligned}\mu =& 0.061749 + 0.00694(S - 17.8) \\ &+ 0.860 \exp\left[1.87\left(\frac{T-20}{20}\right)\right] \\ &+ 0.000611(E - 222) - 0.00080(S - 17.8)^2 \\ &- 0.218 \exp\left[3.74\left(\frac{T-20}{20}\right)\right] \\ &- 0.00000573(E - 222)^2\end{aligned} \quad (2)$$

where μ is the growth rate (d^{-1}), S is salinity (psu), T is temperature (°C), and E is irradiance (μmol photons · $m^{-2} \cdot s^{-1}$). The predictive model fit well ($R^2 = 0.838$) and was significant ($F_{6,33} = 28.47$, $P < 0.001$), but it also had statistically significant lack of fit ($F_{9,24} = 41.98$, $P < 0.001$) resulting from systematic errors at the lowest values of temperature, light, and salinity, where the model overestimates exponential growth rate. The predicted maximum growth rate from equation (2) was 0.94 d^{-1} at a temperature of 27°C, a salinity of 22 psu, and an irradiance of 275 μmol photons · $m^{-2} \cdot s^{-1}$. The maximum empirical growth rate was 0.92 d^{-1} at experimental levels of 29°C, 122 μmol photons · $m^{-2} \cdot s^{-1}$, and 28 psu (Table 2).

To visualize the predictive model for growth rate [eq. (2)], predictions were plotted versus temperature (Fig. 1A). The solid center-point prediction curve shows the predicted growth rates at midpoint values for salinity (17.8 psu) and irradiance (217 μmol photons · $m^{-2} \cdot s^{-1}$). The upper dashed prediction curve shows predicted growth rate in relation to temperature

for optimal salinity (22 psu) and irradiance (275 μmol photons $\cdot m^{-2} \cdot s^{-1}$). This prediction curve represents conditions in relatively well-lit estuarine or coastal areas. The lower dashed prediction curve shows predicted growth rate in relation to temperature for lower salinity (3 psu) and irradiance (100 μmol photons $\cdot m^{-2} \cdot s^{-1}$). These conditions are typical of Texas inland waters during the cooler months in which *P. parvum* blooms (Gandara 2004a–c).

Stationary cell concentration. Similar to exponential growth rate, stationary cell concentration was a unimodal function of temperature (Fig. 1B). Interaction terms were insignificant ($t_{30} < 1.97$, $P > 0.057$) and explained <2% of the variance in stationary cell concentration. The lack of interactions in the predictive model implies that the optimal temperature for stationary cell concentration does not vary significantly with salinity or irradiance. Transformation of stationary cell concentration was necessary due to asymmetry in the response to temperature. The optimized value of the transformation parameter θ was 1.45. The block term was tested with the full model and was significant ($t_{32} = -3.07$, $P < 0.0001$), explaining 8% of the variance in stationary cell concentration. Again, this suggests systematic errors, but the block term was not included in the predictive model since the source of such errors is unknown.

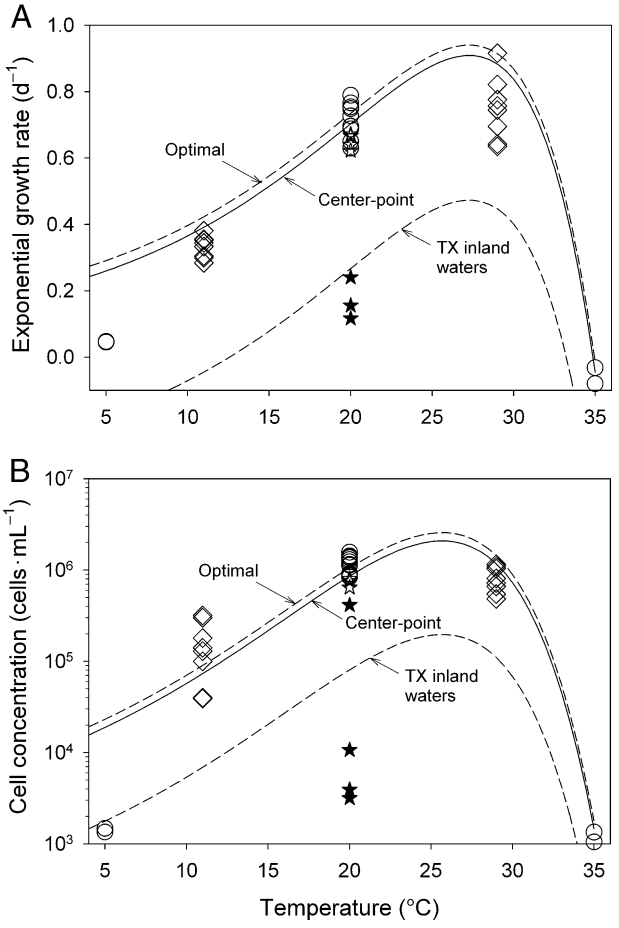

FIG. 1. (A) Exponential growth rate in relation to temperature. Lines show predictions of the regression model (eq. 2) under conditions described in the text. (B) Stationary cell concentration in relation to temperature. Lines show predictions of the regression model (eq. 3) under conditions described in the text. Circles: data from center-point experimental conditions (irradiance of 217 μmol photons $\cdot m^{-2} \cdot s^{-1}$ and salinity of 17.8 psu); diamonds: the factorial points for irradiance and salinity, with all combinations of irradiance equal to 122 or 336 μmol photons $\cdot m^{-2} \cdot s^{-1}$ and with salinity equal to 7.5 or 28 psu; stars: the star points for irradiance and salinity, with filled stars having extremely low salinity (0.5 psu) or irradiance (14 μmol photons $\cdot m^{-2} \cdot s^{-1}$) and open stars having extremely high salinity (35 psu) or irradiance (420 μmol photons $\cdot m^{-2} \cdot s^{-1}$).

The final predictive model had five terms, as follows:

$$\begin{aligned} \ln N = & 6.7519 + 0.0517(S - 17.8) \\ & + 10.33 \exp\left[1.45\left(\frac{T - 20}{20}\right)\right] \\ & + 0.0017(E - 222) - 0.0063(S - 17.8)^2 \\ & - 3.422\left[2.90\left(\frac{T - 20}{20}\right)\right] \end{aligned} \quad (3)$$

where N is stationary cell concentration (cell $\cdot$ mL^{-1}), S is salinity (psu), T is temperature (°C), and E is irradiance (μmol photons $\cdot m^{-2} \cdot s^{-1}$). The model fit reasonably well ($R^2 = 0.6456$) and was significant ($F_{5,34} = 12.39$, $P < 0.001$) but also had statistically significant lack of fit ($F_{10,24} = 21.36$, $P < 0.001$) resulting from systematic errors at the lowest values of temperature, light, and salinity, where the model overestimates stationary cell concentration. The predicted maximal cell concentrations from equation (3) occurred at 26°C and 22 psu and varied directly with irradiance. The maximum cell concentration was empirically measured as 1.57×10^6 cells $\cdot$ mL^{-1} at 20°C, 217 μmol photons $\cdot m^{-2} \cdot s^{-1}$, and 17.8 psu (Table 2).

Similar to growth rate, a visualization of the predictive model was plotted versus temperature (Fig. 1B). The solid center-point prediction line shows the predicted response to temperature at midpoints of salinity (17.8 psu) and irradiance (217 μmol photons $\cdot m^{-2} \cdot s^{-1}$). The upper dashed prediction curve shows stationary cell concentration in relation to temperature for salinity (22 psu) and irradiance (275 μmol photons $\cdot m^{-2} \cdot s^{-1}$) that are optimal for growth. The lower dashed prediction curve shows stationary cell concentration in relation to temperature for lower levels of salinity (3 psu) and irradiance (100 μmol photons $\cdot m^{-2} \cdot s^{-1}$).

Acute toxicity to fish (LC_{50}). Using fathead minnow bioassays, LC_{50} values based on percent dilution were estimated using stationary culture samples. Two cultures, those with the lowest salinity of 0.5 psu, had very low stationary densities and were nontoxic. Five additional cultures with extreme values of temperature and light had an estimated LC_{50} of 50% or greater, an imprecise estimate for the technique used here. Data from these seven cultures were not analyzed further and were censored from the regression anal-

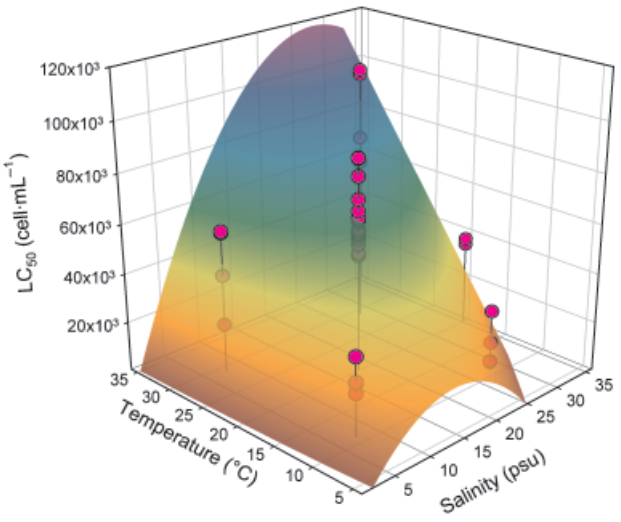

FIG. 2. Acute (48 h) cellular LC_{50} (50% lethal concentration) for juvenile *Pimephales promelas* in relation to salinity and temperature. Circles show data, and the surface shows the fitted regression model (eq. 4).

ysis. For all remaining cultures, a LC_{50} value was calculated using stationary cell concentration to express the cell concentration required to kill 50% of test fish. These estimates were analyzed using multiple regression in relation to the three experimental factors.

Acute toxicity to fish was a complex function of experimental conditions (Fig. 2), with a significant interaction between temperature and salinity ($t_{32} = 4.14$, $P < 0.0003$). The predictive model had four terms, as follows:

$$\begin{aligned} LC_{50} = {} & 62500 + 941(S - 17.8) + 2720(T - 20) \\ & - 172(S - 17.8)^2 + 153(S - 17.8)(T - 20) \end{aligned} \quad (4)$$

where LC_{50} is the cell concentration causing 50% mortality of fish in 48 h (cells $\cdot$ mL^{-1}), S is salinity (psu), and T is temperature (°C). The block term was not significant ($t_{32} = 1.52$, $P = 0.14$) and explained only 2% of the variance in toxic activity. The predictive model fit well ($R^2 = 0.7756$) and was significant ($F_{4,28} = 24.2$, $P < 0.0001$). Lack of fit was insignificant ($F_{8,20} = 0.88$, $P = 0.55$).

A visualization of the predictive model showed a complex response (Fig. 2). Predicted toxicity generally increased as temperature decreased. The predicted unimodal relationship of LC_{50} to salinity implies high toxicity at very low and very high salinities. Minimal toxicity of cells was found near the peak of this unimodal curve, with conditions ranging from 14 psu at 5°C to about 28 psu at 25°C.

DISCUSSION

The exponential growth rate of *P. parvum* was a unimodal function of temperature, lacking any interactions with light or salinity, with rapid growth at temperatures between 25°C and 30°C. These results are similar to those observed for other strains (Larsen et al. 1993, Larsen and Bryant 1998). For example, Larsen et al. (1993) showed a unimodal response for a *P. parvum* strain from Denmark in a range of 10°C–28°C with an optimum of about 0.8 d^{-1} at 26°C (Table 1). Inhibition of *P. parvum* growth at temperatures above 30°C is also consistent with earlier, less quantitative observations (Shilo and Aschner 1953). Comparison with previous studies is difficult because of differences in the temperature levels used. For example, Larsen and Bryant (1998) used four temperatures in a range from 4°C to 30°C but used no temperatures between 15°C and 30°C. However, our observations and those of Larsen et al. (1993) suggest that temperature optima between 25°C and 30°C, as well as growth inhibition at about 10°C, may be common for *P. parvum*.

The exponential growth rate of the Texas strain of *P. parvum* is relatively insensitive to salinity over a wide range, with optimum growth near 22 psu and growth being greatly reduced only below ~1 psu. The salinity optima of other strains were found to range from 8 to 34 psu (Table 1). Although differences in experimental design again complicate comparisons among strains, all strains including the Texas strain grow optimally at salinities greater than those characterizing inland waters with *P. parvum* blooms. Previous studies of light-dependent growth generally suggest increasing and saturating relationships, with the level of saturation ranging from 100 to 200 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} (Table 1). No previous studies report a unimodal relationship as observed here, though more data at high, potentially inhibitory irradiances would be helpful. Such observations could be important for understanding bloom dynamics in the southwestern United States since irradiances are very high near the surface in summer.

A unimodal response to temperature was also seen for stationary cell concentration. Growth and abundance were both highest at relatively warm temperatures (25–28°C) and moderate salinities (20–25 psu). These conditions are not typical of Texas inland waters during *P. parvum* blooms, from autumn through winter to spring (Gandara 2004a–c). Thus, if this strain of *P. parvum* is representative of those now occurring in the southwestern United States, conditions in Texas inland waters apparently are not optimal for growth or abundance at the times of year when blooms occur.

Acute toxicity to juvenile fish (measured as LC_{50} on a cellular basis) was complicated by an interaction of temperature and salinity. The predictive model developed here implies that toxic activity per cell and potentially the production of bioavailable toxins by cells are greatest under conditions that are not optimal for *P. parvum* growth and abundance. Maximum toxicity to fish was observed at extreme (low and high) values of salinity and also for lower temperatures, while maximum growth and abundance were found at warmer temperatures and moderate salinities. Again, for this strain, acute toxicity to fish was increased at conditions suboptimal for growth and abundance, which are also

the conditions under which toxic blooms tend to occur in Texas inland waters.

Acute toxic activity of other harmful algae is often highest under conditions that are suboptimal for growth (Plumley 1997). Although the acute toxicity to fish of *P. parvum* has long been studied (Shilo and Aschner 1953), apparently no previous studies have quantified relationships of such toxicity with temperature, light, and salinity. Previous studies have shown that growth limitation by nutrients enhances hemolytic activity (Dafni et al. 1972, Johansson and Granéli 1999). In this study, reduced growth due to nonoptimal temperature and salinity increased the toxicity of *P. parvum* to juvenile fish. In contrast, previous studies of other strains showed either no consistent effects of temperature, light, or salinity (Larsen and Bryant 1998) or an effect of salinity much weaker than that of phosphate limitation (Larsen et al. 1993), although these studies measured toxicity to *Artemia*, not to fish. Using fish as the test organism can give more realistic information on the potential harm from *P. parvum* blooms, as fish kills are the primary harmful impact for this species.

The results of this study show the dependence of growth, abundance, and toxicity of *P. parvum* under conditions of temperature, salinity, and irradiance ranging from those of coastal oceans to inland waters. Growth and abundance of this strain of *P. parvum* was high under conditions similar to warm, well-lit estuaries, and lower under conditions characterizing blooms in inland waters, with lower temperature, salinity, and irradiance. The design of this study provides its strongest statistical description near the experimental midpoints of these three factors. For example, prediction intervals (Neter et al. 1996) for the center-point of the experimental design indicate likely errors of ~35% for the responses analyzed here, while larger errors of as much as ~50% are likely for the star points. Thus, future studies could focus on the very low salinities (near 1 psu) characterizing inland waters with *P. parvum* blooms, to refine description of its dynamics and toxicity under such conditions. Moreover, the contradiction between high predicted growth and abundance under summer conditions and the occurrence of inland blooms in winter conditions suggests that additional, seasonal factors limit this species. Ecological studies of zooplankton grazing, nutrient limitation, and competition with other phytoplankton could thus be informative.

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